REMARKS

Claims 82-84 and 87-94 are pending in the application. Claims 44-81, 85-86, and 95-96 have been canceled as drawn to a nonelected invention. Claim 82 has been rewritten as an independent claim. Support for the amendments can be found in the original claims as well as throughout the specification, particularly page 29. No new matter has been added by way of amendment. Reexamination and reconsideration of the claims are respectfully requested.

The specification has been amended at page 1 to update the status of priority document U.S. Application No. 09/915,873, now issued as U.S. Patent No. 6,815,184. Applicants wish to make the Examiner aware of copending and commonly owned U.S. Application No. 11/778,480, filed July 16, 2007. The Examiner is respectfully requested to consider this commonly owned invention when examining the claims of the pending application.

The Examiner's comments in the Office Action are addressed below in the order set forth therein.

The specification was objected to because it contains an embedded hyperlink and/or other form of browser executable code. Applicants have amended the specification at pages 13, 18, and 24 to remove the "http://www" at the beginning of the website address, thereby removing the hyperlink and/or browser-executable code. The URL now contained in the specification is consequently not a live weblink such as a hyperlink or browser-executable code. Because this URL is not a hyperlink or browser-executable code as defined in MPEP §608.01, the objection should be withdrawn.

Claim 82 was objected to for depending from method claim 44. Claim 82 has been amended to be an independent claim. Accordingly, the objection to claim 82 should be withdrawn.

Claims 82-96 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant

regards as the invention. The Examiner had indicated that claims 86 and 96, drawn to SEQ ID NO:16 as the 5′ leader sequence, are immune to this rejection. Therefore, as claim 82 has been amended to set forth that SEQ ID NO:16 is the 5′ leader sequence, the rejection of the claims should be withdrawn.

The rejection of the claims under 35 U.S.C. §112, first paragraph, for failing to comply with the written description requirement, and for lack of enablement should be withdrawn. In both instances, the Examiner has indicated that the leader sequence set forth in SEQ ID NO:16 is described and enabled in the specification. Accordingly, as claim, 82 has been amended to set forth that the 5′ leader sequence is SEQ ID NO:16, the rejection should be withdrawn.

Claims 82-96 were also rejected under 35 U.S.C. §112, first paragraph, because the specification is not enabling for the enhanced expression of any biologically active polypeptide in duckweed using the 5′ leader sequence of SEQ ID NO:16. This rejection is respectfully traversed.

The specification provides examples of three diverse polypeptides, α-interferon, human growth hormone, and monoclonal antibodies, and their expression using the 5′ leader sequence set forth in SEQ ID NO:16. Tables 3, 4, and 6, on pages 34, 35, and 37, respectively, of the specification show the expression level of α-2b interferon obtained with the various expression constructs described in Table 2 on page 28 of the specification. The data in these tables demonstrate that very different levels of protein expression are obtained when SEQ ID NO:16 is used as the leader sequence. By substituting in the 5′ leader sequence from the ribulose-bis-phosphate carboxylase small subunit 5B gene of *Lemna gibba* (SEQ ID NO:16) for the 5′-mas leader in pBMPSP3 (see description of IFN053 construct in Table 2), expression level was increased at least another 10-fold beyond that achieved with construct IFN09 (see Table 6, comparing the mean average concentration in the media at the 2 week screening trial for construct IFN09 with that obtained for construct IFN53 at the one week screening trial).

The claimed leader has also been shown to enhance the expression of human growth hormone and monoclonal antibodies in duckweed. See pages 38-39 of the specification,

Examples 5, 6, and 7. See also, page 55 of Gasdaska *et al.* (2003) *Bioprocessing Journal* 2: 49-56 provided as Cite No. 17 in the Information Disclosure Statement filed September 30, 2003.

Applicants have demonstrated that the claimed leader can enhance expression of three diverse polypeptides in duckweed. Thus, it is reasonable to expect, in view of the results demonstrated by Applicants, that the leader would be useful to express other biologically active polypeptides in duckweed. Accordingly, the specification has enabled the use of the 5′ leader sequence set forth in SEQ ID NO:16 for the expression of biologically active polypeptides in duckweed, and the rejection should be withdrawn.

Claims 82-92 were rejected under 35 U.S.C. §103(a) as being unpatentable over Stomp *et al.* (1999, WO 99/07210) further in view of Wong *et al.* (1992, *Plant Molecular Biology* 20:81-93), Buzby *et al.* (1990, *The Plant Cell* 2:805-814), and Stiekema *et al.* (1983, *Nucleic Acids Research* 11:8051-8061). This rejection is traversed as applied to the pending claims.

The present invention is drawn to methods and compositions for the expression and recovery of biologically active recombinant polypeptides, using duckweed as the expression system. One aspect of the present invention provides a method for enhanced expression levels of biologically active polypeptides in duckweed, resulting in an increased polypeptide yield and enabling the production of useful quantities of valuable biologically active polypeptides in this system. The pending claims are drawn to a stably transformed duckweed plant culture or duckweed nodule culture wherein the 5′ leader sequence set forth in SEQ ID NO:16 is used in the transformation construct. The use of SEQ ID NO:16 provides for substantial increases in production of recombinant proteins in duckweed.

Stomp *et al.* provide general teachings regarding methods of modifying nucleotide sequences to increase their expression in duckweed. However, Stomp *et al.* do not teach the use of SEQ ID NO:16, as recited in the pending claims, to enhance expression of a biologically active polypeptide.

Buzby *et al.* is cited as teaching upstream sequences of three BbcS genes from L. gibba, including the 5′ leader sequence from RbcS 5B gene which encompasses SEQ ID NO:16. Wong *et al.* is cited as teaching the 5′ leader from RbcS gene of Arabidopsis.

The Examiner reasons that it would have been obvious to a person with ordinary skill in the art to modify the method of Stomp *et al.* by utilizing the 5′ leader sequence of Buzby *et al.* or Wong *et al.* However, in the rejection of claims 82-96 under 35 U.S.C. §112, first paragraph, for lack of enablement, the Examiner indicated that the specification was only enabling for the 5′ leader sequence of SEQ ID NO:16 for the expression of biologically active polypeptides in duckweed. The Examiner reasoned that the specification did not provide enablement for any 5′ leader sequence or the use of the 5′ leader sequence of SEQ ID NO:16 for enhanced expression of any biologically active polypeptide in duckweed.

In the enablement rejection, the Examiner cited Wong *et al.* for the teaching that the ability of 5′ untranslated leader sequences and translational fusions to increase gene expression is dependent on the coding sequences to which they are attached. Furthermore, the Examiner indicates that it is well known in the art that the effect of a 5′-UTL may vary depending on the plants, particularly between dicots and monocots citing Dai *et al.* Therefore, by the Examiner's argument, it would not have been obvious to combine the references and expect that one would gain enhanced expression. In fact, as the Examiner has noted, it was not predictable in the art that a particular 5′ leader would work, let alone that it would enhance expression.

The specification provides examples of three diverse polypeptides, α-interferon, human growth hormone, and monoclonal antibodies and their expression using the 5′ leader sequence set forth in SEQ ID NO:16. Tables 3, 4, and 6, on pages 34, 35, and 37, respectively, of the specification show the expression level of α-2b interferon obtained with the various expression constructs described in Table 2 on page 28 of the specification. The data in these tables demonstrate that very different levels of protein expression are obtained when SEQ ID NO:16 is used as the leader sequence. By substituting in the 5′ leader sequence from the ribulose-bis-phosphate carboxylase small subunit 5B gene of *Lemna gibba* (SEQ ID NO:16) for the 5′-mas leader in pBMPSP3 (see description of IFN053 construct in Table 2), expression level was increased at least another 10-fold beyond that achieved with construct IFN09 (see Table 6, comparing the mean average concentration in the media at the 2 week screening trial for construct IFN09 with that obtained for construct IFN53). It is important to note that this is **at least a 10-fold <u>further</u> increase** beyond that observed with the combined optimization and

intron modifications of construct IFN09, as the average mean value for construct IFN53 (i.e., 15.3 mg/L) shown in Table 6 is the result of a **one week** growth cycle (compared to a **two week** growth cycle for the other constructs shown in Table 6).

The claimed leader has also been shown to enhance the expression of human growth hormone and monoclonal antibodies in duckweed. See pages 38-39 of the specification, Examples 5, 6, and 7. See also, page 55 of Gasdaska *et al.* (2003) *Bioprocessing Journal* 2: 49-56 provided as Cite No. 17 in the Information Disclosure Statement filed September 30, 2003.

Accordingly, as the claims have been amended to include the 5' leader sequence from the ribulose-bis-phosphate carboxylase small subunit 5B gene of *Lemna gibba* (SEQ ID NO:16), the rejection of the claims under 35 U.S.C. §103 should be withdrawn.

Claims 82-96 were rejected under 35 U.S.C. §103(a) as unpatentable over Stomp *et al.* (1999, WO 99/07210) further in view of Wong *et al.* (1992, *Plant Molecular Biology* 20:81-93), Buzby *et al.* (1990, *The Plant Cell* 2:805-814), Yu *et al.* (1995, U.S. Patent No. 5460952), Park *et al.* (1997, *The Journal of Biological Chemistry* 272:6876-6881) and Stiekema *et al.* (1983, *Nucleic Acids Research* 11:8051-8061). This rejection is traversed as applied to the pending claims.

The Yu reference is cited as teaching a signal peptide for secretion of the protein into the media of the plant cell cultures. Park is cited for the teaching that a signal peptide from rice α-amylase can be recognized and processed by various expression systems. However, neither of the references provide the teachings that the ribulose-bis-phosphate carboxylase small subunit 5B gene of *Lemna gibba* (SEQ ID NO:16) could be used in duckweed to enhance expression of a polypeptide of interest. As discussed above, based on the teachings of the prior art, there was no teaching that SEQ ID NO:16 would work to increase expression of a biologically active polypeptide in duckweed. Accordingly, the rejection of the claims under 35 U.S.C. §103(a) should be withdrawn.

Claims 82-87 were rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 16-17 of U.S. Patent No. 6,815,184 ('184 patent) in

view of Wong *et al.* (1992, *Plant Molecular Biology* 20:81-93) and Buzby *et al.* (1990, *The Plant Cell* 2:805-814). This rejection is respectfully traversed.

As noted above, the claims have been amended to recite that SEQ ID NO:16 is the 5′ leader sequence. The claimed leader has been shown to enhance the expression of α-2b interferon, human growth hormone, and monoclonal antibodies in duckweed. The '184 patent does not teach the use of this leader. Furthermore, neither of the Wong and Buzby references demonstrates that the leader would be useful for expression of biologically active polypeptides in duckweed. Based on the enablement arguments presented by the Examiner, there was no expectation in the art that the claimed leader could be used in duckweed to enhance expression of a biologically active polypeptide. Thus, the rejection of the claims on the ground of nonstatutory obviousness-type double patenting should be withdrawn.

Claims 82-96 were provisionally rejected on the ground of nonstatutory obviousness-type double patenting as unpatentable over claims 3, 8-10, 23, 26-29 of copending Application No. 10/794,615 ('615 application). These are *provisional* rejections because the alleged conflicting claims have not issued as part of a patent.

Applicants respectfully note that the present application and the '615 application are commonly owned. At which time allowable subject has been agreed upon, and a double-patenting rejection over this copending application, or a patent issuing there from, is the only remaining rejection barring allowance of the present application, Applicants will address the filing of a terminal disclaimer.

Claims 82-87 were rejected on the ground of nonstatutory obviousness-type double patenting as unpatentable over claims 1-25 of copending Application No. 10/873,846 ('846 application), in view of Wong *et al.* (1992, *Plant Molecular Biology* 20:81-93), and Buzby *et al.* (1990, *The Plant Cell* 2:805-814). Applicants respectfully note that this application is now abandoned. The subject matter of claims 1-25 is now pending in U.S. Application No. 11/778,480, filed July 16, 2007. This rejection is respectfully traversed as applied to the claims of this copending application.

As noted above, the pending claims have been amended to recite that SEQ ID NO:16 is the 5′ leader sequence. The claimed leader has been shown to enhance the expression of α-2b interferon, human growth hormone, and monoclonal antibodies in duckweed. The '846 application does not teach the use of this leader. Furthermore, as noted above, neither of the Wong and Buzby references demonstrates that the leader would be useful for expression of biologically active polypeptides in duckweed. Based on the enablement arguments presented by the Examiner, there was no expectation in the art that the claimed leader could be used in duckweed to enhance expression of a biologically active polypeptide. Thus, the rejection of the claims on the ground of nonstatutory obviousness-type double patenting should be withdrawn.

CONCLUSION

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

Respectfully submitted,

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